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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: IKU0104PUSA

U S APPLICATION NO. (If known, see 37 C F R 1 5): 09/647705

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP99/01796	5 April 1999 (5.4.99)	4 April 1998 and 8 April 1998

TITLE OF INVENTION: METHOD FOR SEARCHING PHYSIOLOGICALLY ACTIVE SUBSTANCES, PROCESS FOR PRODUCING THESE SUBSTANCES AND DRUGS FOUND BY THE SEARCHING METHOD

APPLICANT(S) FOR DO/EO/US
Kenji Sakamoto

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. X This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ___ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ___ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. X A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. X A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ___ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. X has been transmitted by the International Bureau.
   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. X A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ___ Amendments to the claims of the International Application Under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ___ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ___ have been transmitted by the International Bureau.
   c. ___ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ___ have not been made and will not be made.
8. ___ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ___ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ___ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ___ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ___ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. X A FIRST preliminary amendment.
    ___ A SECOND or SUBSEQUENT preliminary amendment.
14. ___ A substitute specification.
15. ___ A change of power of attorney and/or address letter.
16. ___ Other items or information:

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Date of Deposit: 3 October 1999

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" under 37 C.F.R. 1.10 on the date indicated above and is addressed to: P.O. Box PCT, Commissioner for Patents, United States Patent and Trademark Office, Washington, D.C. 20231

Claire Flood

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/647705	PCT/JP99/01796	IKU0104PUSA

17. X The following fees are submitted:

	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ____ 20 ____ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	17 - 20 =	0	$18.00	$	
Independent claims	5- 3 =	2	X $80.00	$ 80.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$940.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$	
Processing fee of **$130.00** for furnishing the English translation later than ____ 20 ____ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$940.00	
				Amount to be: refunded	$
				charged	$

a. X A check in the amount of $ 940.00 to cover the above fees is enclosed.

b. ____ Please charge my Deposit Account No. 02-3978 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. X The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-3978. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

KENJI SAKAMOTO

Serial No.: To be assigned

Filed: Herewith

For: METHOD FOR SEARCHING PHYSIOLOGICALLY ACTIVE SUBSTANCES, PROCESS FOR PRODUCING THESE SUBSTANCES AND DRUGS FOUND BY THE SEARCHING METHOD

Attorney Docket No.: IKU0104PUSA

I hereby certify that this correspondence is being deposited with the United States Postal Service via Express Mail Label No. EK 631 030 600 US in an envelope addressed to: Commissioner for Patents, BOX PCT, Washington, D.C. 20231 on

3 October 2000 (Date of Deposit) Claire Flood (Signature)

PRELIMINARY AMENDMENT

Box PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the enclosed literal English language translation for this application as follows:

IN THE CLAIMS:

Amend claims 10, 11, 14 and 17 as follows:

Claim 10, line 1, delete "or 9".

Claim 11, line 1, delete "or 9".

Claim 14, line 1, delete "or 13".

Claim 17, line 1, delete "or 16".

REMARKS

The claims have been amended to remove multiple dependencies. If a telephone conference would resolve any questions, such a conference is invited at the convenience of the Examiner or other PTO representative.

Respectfully submitted,

KENJI SAKAMOTO

BROOKS & KUSHMAN P.C.

Dated: October 3, 2000

By _____
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Detailed description of the invention

A Method for searching physiologically active substances and a process for producing same, and drugs found by the searching method.

Technical Field

The invention relates to a method for searching various types of novel physiologically active substances and a process for producing same, and drugs found by the searching method.

Related Art

In the prior art, the searching for unknown physiologically active substances involved analyzing constituents present in body fluids or tissues, identifying and isolating novel substances and investigating the physiological activity of the discovered novel substances.

The method of the prior art described above consists of analyzing constituents present in the body, finding novel substances and investigating their physiological activities. However, the number of constituents present in the body is extremely high, and physiologically active substances are often present only in low concentrations, thereby making finding novel substances a difficult task. Further, because an overwhelming number of physiological reactions take place in the body, and thus, finding the nature of physiological activities the newly found substances have is also difficult. Therefore, finding novel physiologically active substances with the method of the prior art is a difficult task.

Disclosure of the invention

Therefore, one of the objects of the invention is to provide a more efficient method for searching novel physiologically active substances with a certain level of predictability. Furthermore, another one of the objects of the invention is to provide a process for producing the physiologically active substances found by the method described above. In addition, one of the objects of the invention is to provide a novel medicine for treating diabetes, insulin production regulators, gastric acid secretion regulators and growth hormone secretion regulators found by the method of the invention described above.

The inventors have previously invented a more efficient method for searching novel physiologically active substances with a certain level of predictability and filed a patent application (Patent Laid-open Publication No Hei. 10-109997). The method is based on the finding that, among receptors of substances, for which substances or cells having antagonizing effects are present in a body, or, receptors for the Substance A, for which cells or substances having antagonizing effects on cells responding to said Substance A are present in a body, when there exist two or more sizes for the same receptor, the missing portions, i.e. the portions of amino acid sequences which have been spliced, have physiological activities related to said receptor.

The inventors completed the invention after actually verifying that, in performing the searching method of the previously applied patent described above, the method of inferring the amino acid sequences of receptors based on the base sequences of their cDNAs is also valid when two or more cDNA sequences exist for said receptors, and that its is not restricted to the ones based on the amino acid sequences of receptors whose actual existences are known.

Namely, the invention provides a method for searching physiologically active substances comprising, examining a peptide having an amino acid sequence of two or

more sizes for the same receptor by comparing the cDNA sequences of said receptor, wherein the receptor is a receptor of a cell producing an antagonist to a substance present in a body or a receptor of a cell producing an antagonist to said cells *per se*, and then examining which regions of the longer receptor are missing in the shorter receptor by comparing the sequences of the above-mentioned cDNAs. Also, the invention provides a method for producing physiologically active peptides wherein the missing regions, established by the method of the invention described above, or their derivatives are produced. In addition, the invention provides a medicine for treating diabetes comprising, as an active component, a peptide having the effect of increasing the production of insulin by insulin producing cells, said peptide being those successfully searched for and found by the method of the invention described above and having the amino acid sequences indicated by the sequence numbers 1 or 5 of the sequence table, or having amino acid sequences obtained by the substitution inside, deletion from, insertion into or addition to said sequence (number 1 or 5) of one or several amino acids. In addition, the invention provides insulin production regulators comprising, as an active component, a peptide having the effect of regulating the production of insulin by insulin producing cells, said peptides having the amino acid sequence indicated by the sequence number 2 of the sequence table, or having amino acid sequences obtained by the substitution inside, deletion from, insertion into or addition to said sequence (number 2) of one or several amino acids. In addition, the invention provides gastric acid secretion regulators comprising, as an active component, a peptide having the effect of regulating the secretion of gastric acid, said peptides having the amino acid sequences indicated by the sequence numbers 3 or 4 of the sequence table, or having amino acid sequences obtained by the substitution inside, deletion from, insertion into or addition to said sequence (numbers 3 or 4) of one or several amino acids. Growth hormone production regulators are provided, comprising, as an active component, peptides having the effects of regulating the production of hormone, and having the amino acid sequence indicated

by the sequence number 6 of the sequence table or having amino acid sequences obtained by the substitution inside, deletion from, insertion into or addition to said sequence (number 6) of one or several amino acids.

The invention provides a method based on the base sequences of cDNAs, for efficiently searching novel physiologically active substances with a certain level of predictability. In the method of the invention, novel biologically active substances can be found by examining the receptors for substances participating in antagonism, therefore, it is not required as in the prior art, to isolate physiologically active substances present in trace amounts inside biological samples containing extremely large number of constituents. In addition, the physiological activity of the searched and found physiologically active substance is an activity participating in the antagonism described above, therefore, searching for its physiological activity is also considerably easier than that of the prior art. Thus, according to the method of the invention, searching for novel physiologically active substances can be performed with a considerably higher efficiency compared to the related art. Also, the invention provides a novel medicine for treating diabetes having excellent increased effects on insulin production. In addition, novel insulin production regulators, gastric acid secretion regulators and growth hormone production stimulators are provided.

Preferred Embodiments of the Invention

In the method for searching physiologically active substances of the invention, among receptors of cells producing an antagonist to a substance in a body, or among receptors of cells producing an antagonist to said cells *per se*, amino acid sequences of two or more sizes for a same receptor are examined by comparing the cDNA sequences of the receptor and then which region of the longer receptor is missing in

the shorter receptor is examined by comparing the sequences of the above-mentioned cDNAs or the sequences of mature mRNAs.

Namely, the method of the invention concerns cases where two or more receptors with different sizes are generated based on the differences in the base sequences of the mature mRNAs. In other words, the method of the invention searches for splicing variations at the level of the mRNAs. The role of splicing resulting from the splicing variation at the level of mRNAs, which normally does not occur, is not well understood, except that it inactivates gene expression. There are cases where sequences which should originally be translated are consequently not translated, and in contrast, there are cases where sequences which should not be translated are expressed. In most instances, because splicing is not properly performed, even amino acids can not be expressed, but in the other instances, there are actual cases where amino acid sequences generated by splicing or those which are to be deleted function effectively. The method of the invention consists of searching for such splicing variations at the level of mRNAs.

The method of the invention is directed to examining the base sequences of the cDNAs for the above-mentioned receptors and finding those for which cDNAs of the same receptor exist in different sizes. This task can be performed by either determining several times the base sequences and the sizes of the cDNAs for said receptors or, if the information is reported in the literature, by using this information. The glucagon receptor, FGF receptor and GIP receptors can be mentioned as examples of receptors for which two or more cDNAs of the same receptor exist in different sizes.

The physiologically active substances can be obtained by producing peptides identical to the missing sequence regions which have been recognized to contain physiological activity. In most cases, the missing regions consist of relatively short peptides, such that said physiologically active substances may be produced easily by

chemical synthesis using commercially available peptide synthesizers, in such cases. Or, it is possible to produce them using genetic engineering techniques of the art.

The physiological activity of the physiologically active substances obtained participate in the antagonism mentioned above and can therefore be easily verified by suitable methods satisfying the respective antagonistic effects.

In addition, it is well known by those skilled in the art that among peptides having physiological activity in general, there are cases where the physiological activity is maintained even when a small number of amino acids are substituted with other amino acids, a small number of amino acids are added, or a small number of amino acids are deleted. Therefore, also in the scope of the invention is the production of substances having the physiological activities of the physiologically active substances consisting of the missing regions mentioned above, having a small number of amino acids substituted by other amino acids, a small number of amino acids added, or a small number of amino acids deleted, among the amino acids constituting the missing regions mentioned above (in the present application of the invention, such substances are called "derivatives" of the missing regions mentioned above). Preferably, such derivatives have more than 70%, even more preferably 90% homology with the missing regions mentioned above.

The inventors found a peptide having the effect of increased production of insulin by insulin producing cells, using the search method of the invention described above. Since this peptide has the effect of increased production of insulin by insulin producing cells, it is effective as a medicine for treating diabetes, i.e., as mentioned in the embodiments described below, the existence of several types of cDNAs with different sizes for the glucagon receptors is mentioned in literature in the public domain, and by comparing the base sequences of these cDNAs, which regions of the base sequence coding for the longer amino acid sequence are missing from the base sequence coding for the shorter amino acid sequence was examined, and the amino acid sequence of the missing region was inferred (sequence number 1). Then, the

peptide having the amino acid sequence indicated by the sequence number 1 was chemically synthesized and when administered to insulin producing cells, the production of insulin by said cells increased significantly. This led to the knowledge that the peptide having the amino acid sequence indicated by the sequence number 1 is effective as a remedy for diabetes.

In addition, peptides having the amino acid sequence of the sequence number 1 in which one or several amino acids have been substituted, deleted, inserted or added, and having the effect of increasing the production of insulin by insulin producing cells, are also in the scope of the invention. An actual example of such peptides is the peptide having the amino acid sequence represented by the sequence number 5, which was found based on the method of the invention, by comparing the cDNAs for the rat glucagon receptor. In addition, peptides having the effect of increasing the production of insulin, other than the peptides with amino acid sequences represented by the sequence number 1 or 5, should preferably have more than 70%, even more preferably 90% homology with the sequences indicated by the sequence number 1 or 5. Also, while the number of amino acids is not specifically restricted for these peptides, from the perspective of ease of synthesis and antigenicity, about 7 to 20 is preferable, about 7 to 10 is even more preferable. Non-oral administrations such as intravenous injection, intra-muscular injection or enteral administration are preferred for the administration route of the remedy for diabetes of the invention mentioned above. Also, while the dose is suitably determined based on the condition of the patient and the molecular weight of the active principle, 0.01 to 1.0mg per day for 1kg body weight is normally preferred. Further, the remedy can be produced suitably by the methods of the art, and can be used dissolved in physiological saline solutions with a concentration range of 30 to 3000 mg/l, for instance.

In addition, similarly to above, the inventors found a peptide regulating the production of insulin (inhibition or stimulation of production). Its amino acid

sequence is indicated by the sequence number 2. This sequence was found based on the search method of the invention described above, by comparing the cDNA sequences of the Glucose-dependent insuilinotropic polypeptide receptor. When stimulating the production of insulin, similarly to the above, it is effective as a remedy for diabetes. When inhibiting the production of insulin, it is effective as a remedy for hypoglycemia. Peptides effective as insulin production regulators are not restricted to peptides having the amino acid sequence indicated by the sequence number 2, and peptides having the amino acid sequence of the sequence number 2 in which one or several amino acids have been substituted, deleted, inserted or added, and having the effect of regulating the production of insulin by insulin producing cells, are also in the scope of the invention. In such cases, these peptides should preferably have more than 70%, even more preferably 90% homology with the sequences indicated by the sequence number 2. Also, while the number of amino acids is not specifically restricted for these peptides, from the perspective of ease of synthesis and antigenicity, about 25 to 50 is preferable, and about 28 to 35 is even more preferable.

Non-oral administrations such as intravenous injection, intra-muscular injection or enteral administration are preferred for the administration route of the insulin production regulator of the invention mentioned above. Also, while the dose is suitably determined based on the condition of the patient and the molecular weight of the active principle, 0.01 to 1.0mg per day for 1kg body weight is normally preferred. Also, the remedy can be produced suitably by the methods of the art, and can be used dissolved in physiological saline solutions with a concentration range of 30 to 3000 mg/l, for instance.

Furthermore, similarly to above, the inventors found peptides regulating the secretion of gastric acid (inhibition of secretion or stimulation of secretion). Their amino acid sequences are indicated by the sequence numbers 3 and 4. These sequences were found based on the search method of the invention described above, by comparing the cDNA sequences of the gastrin receptor. These peptides, because

they regulate the secretion of gastric acid, are effective as remedies for gastric and duodenal ulcers (in the case of secretion inhibition), or as remedies for the conditions of unsufficient gastric acid secretion (in the case of secretion stimulation). Peptides effective as gastric acid secretion regulators are not restricted to peptides having amino acid sequences indicated by the sequence number 3 or 4, and peptides having the amino acid sequence of the sequence number 3 or 4 in which one or several amino acids have been substituted, deleted, inserted or added, and having the effect of regulating the secretion of gastric acid, are also in the scope of the invention. In such cases, these peptides should preferably have more than 70%, even more preferably 90% homology with the sequences indicated by the sequence number 3 or 4. Also, while the number of amino acids is not specifically restricted for these peptides, from the perspective of ease of synthesis and antigenicity, in the case of sequences indicated by the sequence number 3, about 5 to 20 is preferable, about 5 to 8 is even more preferable, and in the case of sequences indicated by the sequence number 4, about 11 to 30 is preferable, and about 11 to 20 is even more preferable.

Non-oral administrations such as intravenous injection, intra-muscular injection or enteral administration are preferred for the administration route of the gastric acid secretion regulators of the invention mentioned above. Also, while the dose is suitably determined based on the condition of the patient and the molecular weight of the active principle, 0.01 to 1.0mg per day for 1kg body weight is normally preferred. Also, the remedy can be produced suitably by the methods of the art, and can be used dissolved in physiological saline solutions with a concentration range of 30 to 3000 mg/l, for instance.

Furthermore, the inventors found a peptide regulating the production of growth hormones (stimulation of production or inhibition of production). Its amino acid sequence is indicated by the sequence number 6. This peptide, because it can regulate the production of growth hormones, is effective as remedies for dwarfism (in the case of stimulation of production), or as remedies for gigantism (in the case of

inhibition of production). Peptides effective as growth hormone production regulators are not restricted to peptides having the amino acid sequence indicated by the sequence number 6, and peptides having the amino acid sequence of the sequence number 6 in which one or several amino acids have been substituted, deleted, inserted or added, and having the effect of stimulating the secretion of growth hormones, are also in the scope of the invention. In this case, these peptides should preferably have more than 70%, even more preferably 90% homology with the sequences indicated by the sequence number 6. Also, while the number of amino acids is not specifically restricted for these peptides, from the perspective of ease of synthesis and antigenicity, in the case of sequences indicated by the sequence number 6, about 12 to 30 is preferable, and about 12 to 20 is even more preferable.

Non-oral administrations such as intravenous injection, intra-muscular injection or enteral administration are preferred for the administration route of the growth hormone production regulators of the invention mentioned above. Also, while the dose is suitably determined based on the condition of the patient and the molecular weight of the active principle, 0.01 to 1.0mg per day for 1kg body weight is normally preferred. Also, the remedy can be produced suitably by the methods of the art, and can be used dissolved in physiological saline solutions with a concentration range of 30 to 3000 mg/l, for instance.

Examples

In the following examples, the invention will be actually explained. It should be evident that the invention is not limited by these examples.

Example 1 Prediction of the physiologically active peptide from the glucagon receptor.

The amino acid sequence of the rat glucagon receptor described in FEBS Letters 351 (1994) 271-275 has been investigated. The glucagon receptor has four cDNA lengths

reported from the base sequences of the cDNAs, and it is clear that two among them can be translated into amino acids. These are the consequences of a difference in the post-transcriptional splicing, and differ in their mode from the post-translational splicing such as in the case of the calcitonin receptor. However, the inventors predicted that the portion of amino acids generated by the difference in post-translational splicing also have some sort of activity. This amino acid sequence is indicated by the sequence number 1 of the sequence table.

Example 2 Production of the peptide

A commercially available peptide synthesizer was used to synthesize the peptide having the amino acid sequence indicated by the sequence number 2.

Example 3 Stimulating effect on insulin secretion

BxPC-3 cells which are insulin producing cells derived from a human pancreas (source: Dainippon Pharmaceutical Co.,Ltd.), were cultured in RPMI 1640 media containing 10% calf fetal serum, and grown with 5% carbon dioxide gas in a humidified incubator at 37°C. The cells were treated with trypsin prior to seeding with a density of 1 x 10^5/well; when they reached confluency, the peptide of the invention synthesized in Example 2, the peptides A and B, which are unrelated to the invention and whose activities are unknown, as well as a control (physiological saline) were added at 0.01 mg/well, and the cells were cultured for 24 hours. Then, the concentrations of insulin present in the supernatents were measured by colorimetry with an insulin assaying kit (source: Shibayagi Co.,Ltd.). The results are presented in the Table 1 below.

Table 1

samples	insulin concentration (μg/ml)
peptide of the invention	148
control	37.6
peptide A	34.8
peptide B	87.1

As clearly demonstrated in Table 1, the peptide mentioned above, which has been searched for and found with the method of the invention, promotes the production of insulin by the insulin producing BxPC-3 cells. Therefore, the peptide is considered to work effectively for diabetes patients through its insulin synthesizing action.

Example 4 Production of the peptides

A commercially available peptide synthesizer was used to synthesize the peptides having the amino acid sequences indicated by the sequence numbers 1 and 5 which were predicted based on the cDNA sequences of the human glucagon receptor.

Example 5 Stimulating effect on the production of insulin by insulin producing cells

HIT cells, which are insulin producing hamster pancreatic cells, and BxPC-3 cells, which are human pancreatic cells (source: Dai Nippon Seiyaku), were cultured in RPMI 1640 media (source: Dai Nippon Seiyaku) containing 10% calf fetal serum, and grown with 5% carbon dioxide gas in a humidified incubator at 37°C. The cells were treated with trypsin for seeding 96-well culture plates with a density of 1 x 10^4/well; when the cells reached confluency, the media was exchanged to a F12 serum-free media and the cells were cultured for 8 hours. Then, the peptides of the invention produced in Example 4 were dissolved in serum-free RPMI 1640 media; various doses of the peptides indicated by the sequence number 1 for the HIT cells, and various doses of the peptides indicated by the sequence number 5 for the BxPC-3 cells, were added to the wells and the cultures were further maintained for 12 hours. After the cultures have been performed, the stimulatory effects of the peptides of the invention on the production of insulin by the cells were measured by the insulin immunoassay, to seek stimulatory effects on production in comparison to the non-treated groups. Also, the insulin immunoassay, and the calculations for the rates of stimulation for the production of insulin were actually performed as follows. Various

dilutions of the supernatents from the cell culture media were added to the wells coated with immobilized anti-insulin antibodies, and reacted with anti-insulin antibody solutions, according to the protocol of the insulin assay kit commercialized by Shibayagi (Ltd.). The detection was by reacting the biotin labeling the antibodies with the steptavidin-conjugated peroxydase and measuring the coloration catalyzed by the peroxydase. The concentration of insulin was calculated from a standard curve for insulin. When examining the rates of stimulation for the production of insulin in the groups with various doses added, by taking the rate of stimulation for the production of insulin in the reference group with no substance added as 100%, the following was obtained. The results are presented in the Table 2 below.

Table 2

quantity of peptide added (μg/well)	rate of stimulation for the production of insulin (%)	
	HIT cells	BxPC-3 cells
0	100.0	100.0
0.01	102.8	110.9
0.1	131.6	123.3
1	184.9	198.2
10	203.1	181.1

As clearly demonstrated in Table 2, the peptides mentioned above, which have been searched for and found with the method of the invention, have the effect on the insulin producing cells derived from hamster and human, of stimulating the production of insulin. Therefore, the peptides are considered as leading to a decrease in the level of blood sugar and thereby are useful for the treatment of diabetes.

Example 6 Production of peptides

A commercially available peptide synthesizer was used to synthesize the peptides having the amino acid sequences indicated by the sequence numbers 3 and 4 which were predicted by comparing the cDNA sequences of the gastrin receptor.

Example 7 Inhibitory effect on the secretion of gastric acid in rats

The inhibitory effect on the secretion of gastric acid was evaluated using rats (Wister line), by dissolving in physiological saline, the peptides synthesized in Example 6 and gastrin, a stimulator of gastric acid secretion, hypodermically injecting the previous solution, and measuring the secretion of gastric acid 10 minutes later. Gastrin was administered to obtain a dose of 4μg/kg and the peptides of the invention were administered to obtain a dose of 10μg/kg. The rats were immobilized on a fixator, their gastric juice was sampled by inserting a probe through the mouth into the stomach, and the pH was measured to evaluate the secretion of gastric acid. When the secretion of gastric acid was compared between the reference group with no substance administered and the administered group, the following was obtained for the inhibition on the secretion of gastric acid. The results are presented in the Table 3 below.

Table 3

	Secretion of gastric acid (pH)	
	Sequence No. 3	Sequence No. 4
Control	3.2	3.1
administered group	5.3	4.8

As clearly demonstrated in Table 3, the peptides mentioned above, which have been searched for and found with the method of the invention, have the effect of inhibiting the secretion of gastric acid in rats. Therefore, the peptides are useful a gastric and duodenal ulcer remedies which inhibit secretion of gastric acid.

Example 8 Production of the peptide

A peptide having the amino acid sequence indicated by the sequence number 6, which was predicted by comparing the amino acid sequences of somatostatin receptor, was synthesized with a commercially available peptide synthesizer.

Example 9 Stimulation of the production of growth hormone by growth hormone producing cells

Hamster-derived GH3 cells (source: IFO, deposition No. 50105), which are growth hormone-producing hypophyseal cells, were grown in an F12 media (source: Dai Nippon Seiyaku) containing 15% calf fetal serum, and grown with 5% carbon dioxide gas in a humidified incubator at 37°C. The cells were treated with trypsin for seeding 96-well culture plates with a density of 1 x 10^4/well; when the cells reached confluency, the media was exchanged to a 15% serum F12 media and the cells were cultured for 12 hours. Then, the peptides of the invention produced in Example 7 were dissolved in 15% serum F12 media to add various doses of the peptides to the wells, and the cultures were further maintained for 3 days. After the cultures have been performed, the stimulatory effects of the peptides on the production of growth hormones by the cells were measured by the growth hormone immunoassay, to examine stimulatory the effects on the production, in comparison with the non-treated groups. Also, the growth hormone immunoassay was performed with a method of the art, the sandwich ELISA, i.e., various dilutions of the supernatents from the cell culture media were added to wells coated with immobilized anti-growth hormone antibodies, and reacted for 180 minutes at room temperature, washed before further addition of anti-growth hormone antibodies labeled with peroxydase, and reacted for 60 minutes at room temperature. After washing, the coloring reaction catalyzed by the peroxydase was measured by measuring the absorbance. The concentration of growth hormone was examined based on standard curves obtained by performing the same sandwich ELISA using already known concentrations of growth hormone. When examining the rates of stimulation for the production of growth hormones in the groups with various doses added, by taking the rate of stimulation for the production of growth hormone for the reference group with no peptides added as 100%, the following was obtained. The results are presented in the Table 4 below.

Table 4

quantity of peptide added (μg/well)	stimulation rate of growth hormone production (%)
0	100.0
0.01	101.2
0.1	124.1
1	175.6
10	190.4

As clearly demonstrated in Table 4, the peptide synthesized in Example 8 have the effect on the growth hormone producing cells of stimulating the production of growth hormones. Therefore, the peptides are considered to lead to the stimulation of growth, and therefore useful as a remedy for dwarfism.

What is claimed is

1. A method for searching physiologically active substances comprising; examining a receptor having an amino acid sequence having two or more sizes for the same receptor by comparing a cDNA sequence of said receptor, wherein the receptor being a receptor of a cell producing an antagonist to substance in a body or a receptor of a cell producing an antagonist to said dell *per se;* and examining which region of the longer receptor are missing in the shorter receptor by comparing the sequences of the cDNAs.

2. A method of producing physiologically active peptides, wherein the missing region determined by the method of claim 1, or its derivatives, are produced.

3. A method of claim 2, wherein the missing region is produced.

4. A method of claim 3, wherein the missing region is synthesized by chemical synthesis.

5. A medicine for treating diabetes comprising as an active component a peptide having the effect of increased production of insulin by insulin producing cells, the peptide having the amino acid sequences indicated by the sequence numbers 1 or 5 of the sequence table or having amino acid sequences obtained by the substitution inside, deletion from, insertion into or addition to said sequences of one or several amino acids.

6. A medicine for treating diabetes of claim 5 comprising as an active component a peptide having the amino acid sequence indicated by the sequence number 1 of the sequence table.

7. A medicine for treating diabetes of claim 5 comprising as an active component a peptide having the amino acid sequence indicated by the sequence number 5 of the sequence table.

8. An insulin production regulator comprising as an active component a peptide having the effect of regulated production of insulin by insulin producing cells, the peptide having the amino acid sequence indicated by the sequence number 2 of the sequence table or amino acid sequences obtained by the substitution inside, deletion from, insertion into or addition to said sequence of one or several amino acids.

9. The insulin production regulator of claim 8 comprising as an active component a peptide having the amino acid sequence indicated by the sequence number 2.

10. The insulin production regulator of claim 8 or 9, the insulin production regulator being an insulin production inhibitor.

11. The insulin production regulator of claim 8 or 9, the insulin production regulator being a medicine for treating diabetes.

12. A gastric acid secretion regulator comprising as an active component a peptide having the effect of regulating the secretion of gastric acid, the peptide having the amino acid sequences indicated by the sequence numbers 3 or 4 of the sequence table or amino acid sequences obtained by the substitution inside, deletion from, insertion into or addition to said sequences of one or several amino acids..

13. The gastric acid secretion regulator of claim 12 comprising as an active component a peptide having the amino acid sequences indicated by the sequence numbers 3 or 4 of the sequence table.

14. The gastric acid secretion regulator of claim 12 or 13, the gastric acid secretion regulator being an inhibitor of gastric acid secretion.

15. A growth hormone production regulator comprising as an active component a peptide having the effect of regulating the production of growth hormone, the peptide having the amino acid sequences indicated by the sequence number 6 of the sequence table or having amino acid sequences obtained by the substitution inside, deletion from, insertion into or addition to said sequences of one or several amino acids.

16. The growth hormone production regulator of claim 15 comprising as an active component, a peptide having the amino acid sequence indicated by the sequence number 6 of the sequence table.

17. The growth hormone production regulator of claim 15 or 16, the growth hormone production regulator being a stimulator of growth hormone production.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee: Kenji Sakamoto
Serial or Patent No.: PCT/JP99/01796
Filed or Issued: April 5, 1999 Attorney Docket No. IKU0104PUSA
For: METHOD FOR SEARCHING PHYSIOLOGICALLY ACTIVE SUBSTANCES, PROCESS FOR PRODUCING THESE SUBSTANCES AND DRUGS FOUND BY THE SEARCHING METHOD

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS

I hereby declare that I qualify as a small entity for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled:
METHOD FOR SEARCHING PHYSIOLOGICALLY ACTIVE SUBSTANCES, PROCESS FOR PRODUCING THESE SUBSTANCES AND DRUGS FOUND BY THE SEARCHING METHOD
and described in:

___ the specification filed herewith
X application Serial No. PCT/JP99/01796, filed April 5, 1999.
___ Patent No. _____, issued _____.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 C.F.R. § 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d) or a non-profit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[x] no such person, concern, or organization
[ ] persons, concerns or organizations listed below*

** Note:* Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27).

NAME Hideo Nakoshi
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Hideo Nakoshi
Name of Individual

Hideo Nakoshi
Signature of Individual

Oct 23, 2000
Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee: Kenji Sakamoto
Serial or Patent No.: PCT/JP99/01796
Filed or Issued: April 5, 1999 Attorney Docket No. IKU0104PUSA
For: METHOD FOR SEARCHING PHYSIOLOGICALLY ACTIVE SUBSTANCES, PROCESS FOR PRODUCING THESE SUBSTANCES AND DRUGS FOUND BY THE SEARCHING METHOD

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§ 1.9(f) and 1.27(b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 C.F.R. § 1.9(c) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled:
METHOD FOR SEARCHING PHYSIOLOGICALLY ACTIVE SUBSTANCES, PROCESS FOR PRODUCING THESE SUBSTANCES AND DRUGS FOUND BY THE SEARCHING METHOD
and described in:

___ the specification filed herewith
X application Serial No. PCT/JP99/01796, filed April 5, 1999.
___ Patent No. _____, issued _____.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 C.F.R. § 1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d) or a non-profit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[ ] no such person, concern, or organization
[x] persons, concerns or organizations listed below*

** Note:* Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27).

NAME Hideo Nakoshi
ADDRESS 10-9-103 Omaru, Tsuzuki-ku, Yokohama-shi, Kanagawa, 224-0061 Japan
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ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NON-PROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Kenji Sakamoto		
Name of Inventor	Name of Inventor	Name of Inventor
[signature]		
Signature of Inventor	Signature of Inventor	Signature of Inventor
Oct 23, 2000		
Date	Date	Date

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

Atty. Docket No. IKU 0104 PUSA
First Named Inventor Kenji Sakamoto

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR SEARCHING PHYSIOLOGICALLY ACTIVE SUBSTANCES, PROCESS FOR PRODUCING THESE SUBSTANCES AND DRUGS FOUND BY THE SEARCHING METHOD

the specification of which:

[ ] is attached hereto; or

[X] was filed on (MM/DD/YYYY) 04/05/1999 as U.S. Application Number or PCT International Application Number PCT/JP99/01796, and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	*Country*	*Foreign Priority Date (MM/DD/YYYY)*	*Priority Not Claimed*	*Certified Copy Attached? (Yes/No)*
Hei 10 - 108662	JP	04/04/1998		No
Hei 10 - 112819	JP	04/08/1998		No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

Application Number(s)	*Filing Date (MM/DD/YYYY)*

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

Application Number(s)	*Filing Date (MM/DD/YYYY)*	*Status: Patented, Pending, Abandoned*

Declaration for Patent Application (cont'd.) Atty. Docket No. IKU 0104 PUSA

I hereby appoint the following registered practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of Sole or First Inventor Kenji Sakamoto

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B&K0001/9801 [Decl. -- Page 2 of 2]

TOTAL P.05